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Signed this 26th day of October 2001

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# PATENT OF INVENTION

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<p><b>3 TITLE OF THE INVENTION (not more than 200 characters)</b></p> <p><b>AN EDIBLE EMULSION INCLUDING LIVE MICROORGANISMS, AND A DRESSING SAUCE OR VINAIGRETTE INCLUDING SAID EDIBLE EMULSION</b></p>			
<p><b>4 PRIORITY CLAIM OR REQUEST FOR THE BENEFIT OF THE FILING DATE OF A FRENCH PRIOR APPLICATION</b></p>		<p>Country or organization Date ____ / ____ / ____ No. _____</p> <p>Country or organization Date ____ / ____ / ____ No. _____</p> <p>Country or organization Date ____ / ____ / ____ No. _____</p> <p><input type="checkbox"/> If there are any other priorities, please check in the box and use the « Continuation » form</p>	
<p><b>5 APPLICANT</b></p>		<p><input type="checkbox"/> If there are any other applicants, please check in the box and use the « Continuation » form</p>	
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<b>7 INVENTOR(S)</b>			
The inventors are the applicants		<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No If the answer is no, provide a separate designation of inventor(s)	
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**DESIGNATION OF INVENTOR(S) Page No. 1/1**

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Your references for this file: (optional)		<b>B0075/FR</b>	
NATIONAL REGISTRATION NO.		<b>FR-00 13101</b>	
TITLE OF THE INVENTION (not more than 200 characters)  <b>AN EDIBLE EMULSION INCLUDING LIVE MICROORGANISMS, AND A DRESSING SAUCE OR VINAIGRETTE INCLUDING SAID EDIBLE EMULSION</b>			
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## DOCUMENT CONTAINING MODIFICATIONS

PAGE(S) OF DESCRIPTION OR OF CLAIMS OR OF DRAWINGS			A.C.	Date of correspondence	DATING STAMP OF CORRECTOR
Amended	Deleted	Added			
p15			X	Dec 21, 2000	EMI Dec 28, 2000

Any change made to the original claims, unless it is based on the provision of Article 28 of the Decree of September 19, 1979, shall be indicated by the mention "A.C." (Amended Claims).

AN EDIBLE EMULSION INCLUDING LIVE MICROORGANISMS, AND A  
DRESSING SAUCE OR VINAIGRETTE INCLUDING SAID EDIBLE  
EMULSION

The present invention relates to edible  
5 water/oil/water emulsions including an inner water phase  
itself containing live microorganisms in sufficient  
quantity to confer specific nutritional properties on the  
edible emulsion.

The present invention relates to an edible  
10 water/oil/water emulsion comprising:

- an inner aqueous phase containing live micro-organisms;
- a lipid phase for protecting the microorganisms, said inner aqueous phase and the lipid phase forming a  
15 primary water/oil emulsion; and
- a conserving outer aqueous phase in which the primary emulsion is dispersed.

It is already known that edible water/oil/water emulsions can be made using live microorganisms in the  
20 inner aqueous phase of an edible water/oil/water emulsion. The only known applications relate to milk products or derivatives thereof forming a water/oil/water emulsion in which the inner aqueous phase contains active bacterial bodies, the outer aqueous phase being formed by  
25 a milk product which may optionally be fermented.

All available information on the subject of active bacterial bodies or live microorganisms used in edible emulsions of the above-mentioned milk type relates to using bacteria of the bifidus family which, because of  
30 its properties and its wide availability, is a live microorganism whose use is very widespread in the food industry.

The principle on which making such water/oil/water emulsions containing live microorganisms is based, is  
35 that of being able to benefit from the presence of live microorganisms which confer specific properties and organoleptic quality to the emulsion even though the live

microorganisms would be destroyed if they were to come into contact with ingredients in the outer aqueous phase (organic acids) which confer its organoleptic properties on the emulsion (or the sauce in general). This raises the problem of conserving live microorganisms in such emulsions or sauces.

One of the frequently-used techniques has thus consisted in seeking out and selecting bifidus type bacteria that present particularly high resistance to the medium or the outer aqueous phase in which the bacteria are to be conserved. Such techniques are not generally applicable and they can be extremely limiting, while also being of a cost and requiring implementation times that are often incompatible with industrial requirements, which means that those techniques are in rather limited use.

It has also been envisaged that the state of the microorganisms, in particular of bifidus bacteria, could be modified so as to obtain them in a dry or frozen form, after adding a stabilizer thereto. Other methods have consisted in attempting to obtain bifidus bacteria in powder form so as to increase ability to withstand an acid medium. Tests have also been reported seeking to freeze bifidus bacteria or to obtain a granular state, always for the purpose of increasing resistance specifically to acid media.

Together, those techniques appear to be unsuitable for generalizing to any given situation or to any type of live microorganism, or indeed to edible water/oil/water emulsions other than milk products.

The present invention thus seeks to propose a novel edible water/oil/water emulsion that does not present the above-mentioned drawbacks but which, on the contrary, is capable of containing a sufficient quantity of live microorganisms to confer particular nutritional properties and taste to the emulsion, while also ensuring



that the water/oil/water emulsion presents sufficient acidity to enable it to be used as a dressing sauce.

Another object of the invention is to provide a novel water/oil/water emulsion capable of encouraging  
5 good conservation of live microorganisms in its inner aqueous phase.

Another object of the invention is to provide a novel edible water/oil/water emulsion which is not limited to using a particular type or form of live micro-  
10 organism.

Another object of the invention is to provide a novel edible water/oil/water emulsion that is particularly simple to make, using conventional basic ingredients, in particular as media for the live micro-  
15 organisms.

Another object of the invention is to provide a dressing sauce or vinaigrette, in particular for salad, that includes live microorganisms and that possesses nutritional and organoleptic qualities of particular  
20 interest.

These objects assigned to the invention are achieved by means of an edible water/oil/water emulsion comprising:

- an inner aqueous phase containing live micro-  
25 organisms;
  - a lipid phase for protecting the microorganisms, said inner aqueous phase and the lipid phase forming a primary water/oil emulsion; and
  - a conserving outer aqueous phase in which the  
30 primary emulsion is dispersed;
- the emulsion being characterized in that the outer aqueous phase contains 0.1% to 9% organic acid.

The objects assigned to the invention are also achieved by means of a dressing sauce or vinaigrette, in  
35 particular for salads, comprising an edible water/oil/water emulsion comprising:

- an inner aqueous phase containing live microorganisms;

- a lipid phase for protecting the microorganisms, said inner aqueous phase and the lipid phase forming a primary emulsion; and

- a conserving outer aqueous phase in which the primary emulsion is dispersed;

the emulsion being characterized in that the outer aqueous phase contains 0.1% to 9% organic acid.

10 The objects assigned to the invention will be better understood in the light of the description, tables, and implementations described below, given purely as non-limiting and illustrative examples.

15 The edible water/oil/water emulsion of the invention comprises an inner aqueous phase containing live microorganisms, a lipid phase for protecting the microorganisms, said inner aqueous phase and the lipid phase being intended to be emulsified together to form primary emulsion. The edible water/oil/water emulsion of the  
20 invention also comprises a conserving outer aqueous phase in which the above-defined primary emulsion is subsequently dispersed.

The edible water/oil/water emulsion of the invention is more particularly, but not exclusively, intended for  
25 making a dressing sauce or vinaigrette, in particular for salad, meat, or fish, that can present a variety of rheological states, e.g. liquid, creamy, semi-liquid, or semi-solid states.

In the invention, the inner aqueous phase contains  
30 the live active ingredients, specifically microorganisms which, in non-limiting manner, can be based on lactic ferments, lactic yeasts, or lactic bacteria as such, or it can be based on a simple or complex mixture of any one of said ferments, yeast, or bacteria.

35 In the invention, the inner aqueous phase is the phase of the final edible emulsion that provides its special features and particular organoleptic and

nutritional qualities due to the presence of the live microorganisms, and it is this phase which constitutes the medium in which the microorganisms live. In the invention, the inner aqueous phase is advantageously  
5 constituted by a mixture of water and a medium containing the microorganisms. In a particularly advantageous version of the invention, the medium is constituted by yogurt or cream cheese.

10 In order to preserve and conserve an acceptable quantity of live microorganisms, for example not less than  $10^6$  bacteria per gram (g) of sauce, the acidity of the inner aqueous phase must not exceed a maximum acidity level of 1.5% acetic acid equivalent in the emulsion of the invention.

15 In advantageous and non-limiting manner, microorganisms suitable for use in the aqueous phase of the invention are selected from the following strains:  
Lactobacillus bulgaricus, Lactobacillus casei,  
Lactobacillus acidophilus, Lactococcus lactis,  
20 Streptococcus thermophilus, Bifidobacterium bifidum, and Bifidobacterium lingum.

The lipid phase for protecting the microorganisms serves essentially to protect the microorganisms against attack, and in particular against the acidity of the  
25 outer aqueous phase, while nevertheless allowing the primary emulsion to be made out of the inner aqueous phase and the lipid phase itself. Thus, the protective lipid phase is based on oil, in particular vegetable oil, and a lipophilic emulsifier to make a water in oil  
30 emulsion with the inner aqueous phase. Emulsification operations are implemented using conventional techniques well known to the person skilled in the art.

Advantageously, a primary emulsion presenting improved characteristics is obtained when the weight  
35 ratio of the inner aqueous phase to the lipid phase lies in the range 1/1 to 1/4, and preferably in the range 15/25 to 15/60, i.e. preferably in the range 0.6 to 0.25.

The inner aqueous phase and the lipid phase can be mixed together using a high-shear emulsification technique while nevertheless obtaining a fine emulsion so that the size of the droplets in the primary emulsion is less than or equal to 5 microns, and preferably less than or equal to 1 micron.

The edible water/oil/water emulsion of the invention also has a conserving outer aqueous phase in which the primary emulsion is dispersed, said outer phase having the general function of conserving the emulsion and of providing specific organoleptic character, i.e. taste in the final edible emulsion. For this purpose, the outer aqueous phase contains organic acids for giving it its own taste and flavor, which organic acids can comprise singly or in mixtures, and in non-limiting manner: acetic acid; citric acid; maleic acid; tartaric acid; gluconic acid; or lactic acid.

The organic acids give the final edible emulsion acidity to which the live microorganisms are particularly sensitive, though it is essential to limit the concentration of organic acid, although without having a negative effect on the flavor or the taste, and in general, on the organoleptic properties of the final edible emulsion.

In the invention, it has been found and shown that the outer aqueous phase of the edible emulsion should contain 0.1% to 9% organic acids to obtain good conservation over time of the product without undesirable germs developing, while also obtaining an edible emulsion of taste, nature, flavor, and organoleptic properties that are satisfactory, and ensuring that the microorganisms remain live in the inner phase.

Depending on the intended application, the outer aqueous phase can also include a hydrophilic emulsifier, or even yogurt to obtain an interesting variation in taste, particularly in applications relating to dressing sauces or vinaigrettes, in particular for salads.

The outer aqueous phase and the primary emulsion are mixed together by any technique and method well known to the person skilled in the art, for example slow emulsification of the Stephan type, preferably while  
5 cold, and while ensuring that the final emulsion has droplets of a size lying in the range 10 microns ( $\mu\text{m}$ ) to 200 microns, and preferably in the range 50 microns to 100 microns.

It is also possible to envisage making an outer  
10 aqueous phase which is obtained by a method that involves a cooking operation.

In general, care should be taken to make a final edible water/oil/water emulsion in which the weight ratio of primary emulsion to outer aqueous phase lies in the  
15 range 80/20 to 40/60, i.e. 4 to 0.666.

In practice, care should be taken to obtain a final edible emulsion in which the lipid phase is always present in a quantity which is greater than that of the inner aqueous phase, the content of lipid phase in the  
20 final edible emulsion being ideally close to 25%, in spite of the difficulty in obtaining this threshold.

Preferred applications of the invention seek to provide a water/oil/water emulsion constituted by a vinaigrette for dressing salads. Other preferred  
25 applications of the invention relate to dressing sauces having a lower oil content.

In either case, dressing sauces or vinaigrettes, in particular for salads, will also include seasoning ingredients mixed in the water/oil/water emulsion. Under  
30 such circumstances, the seasoning ingredients can be in liquid or semi-liquid form and can be based on mustard, herbs, or flavorings (garlic, onions).

#### Example 1

35 In this series of examples, Test 1 is an edible water/oil/water emulsion of the invention while Test 2

corresponds to a simple water/oil emulsion without any outer aqueous phase.

In Test 1, the general yogurt taste of the emulsions in question was provided by the yogurt outer aqueous phase which is pasteurized.

The various phases were mixed using conventional slow emulsification techniques of the Stephan type.

TABLE 1

10

Example 1		
	Test 1 (W/O/W)	Test 2 (W/O)
1 - Inner aqueous phase		
Water	3.50	16.84
Sugar	1.00	
Salt	0.00	1.00
Yogurt	10.00	20.00
Alcohol vinegar	0.00	3.00
Xanthan		0.15
<b>Subtotal</b>	<b>14.50</b>	<b>41.00</b>
2 - Lipid phase		
Sunflower oil	5860	58.60
PRPG (lipophilic emulsifier)	0.40	0.40
<b>Subtotal</b>	<b>59.00</b>	<b>59.00</b>
3 - Outer aqueous phase		
10° alcohol vinegar	3.00	
Salt	0.50	
Water	12.65	
Tween 60 (hydrophilic emulsifier)	0.20	
Xanthan	0.15	
Yogurt	10.00	
<b>Subtotal</b>	<b>26.50</b>	
<b>TOTAL</b>	<b>100.00</b>	<b>100.00</b>
Quantity of live germs:		
Lactobacillus bulgaricus + Streptococcus thermophilus		
at t0	1.00×10 <sup>7</sup>	4.00×10 <sup>7</sup>
at t+1 months at 8°C	5.10×10 <sup>7</sup>	6.20×10 <sup>7</sup>
at t+2 months at 8°C	7.00×10 <sup>7</sup>	1.00×10 <sup>4</sup>

The quantity of live bacteria in the emulsions during storage at 8°C was monitored at regular intervals, i.e. at 1 month and at 2 months after the initial emulsions had been made.

15

Comparative analysis of the results of the tests show that by 2 months, the microorganisms in a W/O emulsion had been progressively destroyed while at 2 months the microorganisms in a W/O/W emulsion had been conserved in spite of the significant presence of acid in the outer aqueous phase due to the vinegar.

TABLE 2

	Test 1	Test 2
pH	3.50	3.10
Total acidity (ac)	0.30	0.32
Dry extract (%)	61.60	61.00

10

For each of Tests 1 and 2, above Table 2 gives the general physico-chemical characteristics of the resulting edible emulsions.

#### 15 Example 2 (Table 3)

In this series of tests, Tests A and B correspond to edible water/oil/water emulsions of the invention while Test C corresponds to a simple oil/water emulsion without an inner aqueous phase.

20 In each of the examples, the general yogurt taste of the emulsions in question was provided by the yogurt outer aqueous phase.

The various phases were mixed using conventional emulsification techniques of the Stephan type.

25 It can be seen that the main differences between Tests A and B relate to the quantity of vinegar in the outer phase which leads to an increase in acidity for Test B compared with Test A.

30 Comparative analysis of the emulsions made in this way shows that in Test C (which does not correspond to the invention) a significant quantity of microorganisms were destroyed within 1 month of the emulsion being made.

In contrast, it can be seen that in the examples corresponding to the invention (Tests A and B) the micro-

organism strains are conserved differently 1 month after the emulsion was made, with better conservation being shown for Test A which had less acid in its outer phase.

5 This demonstrates the negative influence of increasing the acidity of the outer aqueous phase, thus revealing that the lipid protection phase is partially permeable, having the result in Test B of strains being destroyed, whereas in Test A (3% 10° alcohol vinegar in the outer phase) good results were obtained.

10 This example thus shows the protective effect in terms of microorganism conservation of the intermediate oily membrane formed by the lipid phase.

15 The quality of live microorganisms was monitored in the product stored at 8°C at time t0 and 1 month after the final emulsions had been made, and this was done using two distinct measurements so as to reduce the margins of error.



TABLE 3

Example 2			
	Test A	Test B	Test C
Type of emulsion	(W/O/W)	(W/O/W)	(O/W)
1 - Inner aqueous phase			
Water	3.50	3.50	
Sugar	1.00	1.00	
Yogurt	10.00	10.00	
<b>Subtotal</b>	<b>14.50</b>	<b>14.50</b>	<b>0.00</b>
2 - Lipid phase			
Sunflower oil	58.60	58.60	60.00
PRPG (lipophilic emulsifier)	0.40	0.40	
<b>Subtotal</b>	<b>59.00</b>	<b>59.00</b>	<b>60.00</b>
3 - Outer aqueous phase			
10° alcohol vinegar	3.00	9.00	3.00
Sugar	0.00	0.00	1.00
Salt	0.50	0.50	0.50
Water	12.65	6.65	15.15
Tween 60 (hydrophilic emulsifier)	0.20	0.20	0.20
Xanthan	0.15	0.15	0.15
Yogurt	10.00	10.00	20.00
<b>Subtotal</b>	<b>26.50</b>	<b>26.50</b>	<b>40.00</b>
<b>TOTAL</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>
4 - Analysis			
pH	3.55	3.33	3.47
Total acidity (%)	0.44	1.12	0.48
Salt (%)	0.53	0.56	0.59
Dry extract	63.80	63.40	62.30
5 - Microbiological analysis at t0 (germs/g)			
(LB + ST)* 1st measurement	$7.00 \times 10^6$	$1.40 \times 10^6$	$3.30 \times 10^7$
(LB + ST)* 2nd measurement	$5.00 \times 10^6$	$1.00 \times 10^6$	$3.30 \times 10^7$
Total mesophilic germs	<10	<10	10
Yeasts	<50	<50	<50
Molds	<50	<50	
Fec. coli.	<10	<10	<10
6 - Microbiological analysis at 1 month at 8°C (germs/g)			
(LB + ST)* 1st measurement	$1.30 \times 10^3$	20	10
(LB + ST)* 2nd measurement	$1.50 \times 10^4$	70	$5.00 \times 10^2$

\*: LB = Lactobacillus bulgaricus  
ST = Streptococcus thermophilus

5

The tests performed thus lead to the conclusion that it is possible to make edible water/oil/water emulsions that initially contain live microorganisms, said edible

water/oil/water emulsions being generally acidic in character and having nutritional properties associated with the presence of organic acids, providing the live microorganisms are protected by a protective lipid phase and providing the organic acid content and the acidity of the inner aqueous phase are limited. These dispositions then make it possible to extend the activity of the microorganisms for a sufficient length of time and thus to obtain an edible water/oil/water emulsion with marked nutritional and organoleptic qualities and properties.

## CLAIMS

1/ An edible water/oil/water emulsion comprising:

- an inner aqueous phase containing live microorganisms;

5       - a lipid phase for protecting the microorganisms, said inner aqueous phase and the lipid phase forming a primary water/oil emulsion; and

- a conserving outer aqueous phase in which the primary emulsion is dispersed;

10       the emulsion being characterized in that the outer aqueous phase contains 0.1% to 9% organic acid.

2/ An emulsion according to claim 1, characterized in that the inner aqueous phase presents maximum acidity of  
15       less than 1.5% acetic acid equivalent.

3/ An emulsion according to claim 1 or claim 2, characterized in that the inner aqueous phase contains microorganisms based on lactic ferments, on lactic  
20       yeasts, or on lactic bacteria, optionally in a mixture.

4/ An emulsion according to claim 3, characterized in that the inner aqueous phase is formed by a mixture of water and a medium containing the microorganisms.  
25

5/ An emulsion according to claim 4, characterized in that the medium is formed by yogurt or cream cheese.

6/ An emulsion according to any one of claims 1 to 5, characterized in that the microorganisms are selected  
30       from the following strains: *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lacococcus lactis*, *Streptococcus thermophilus*, *Bifidobacterium bifidum*, and *Bifidobacterium lingum*.

35

7/ An emulsion according to any one of claims 1 to 6, characterized in that the lipid phase is based on

vegetable oil and a lipophilic emulsifier to make a water in oil emulsion with the inner aqueous phase.

5        8/ An emulsion according to any one of claims 1 to 7,  
characterized in that the weight ratio of inner aqueous  
phase to lipid phase lies in the range 1/1 to 1/4, and  
preferably in the range 15/25 to 15/60.

10       9/ An emulsion according to any one of claims 1 to 8,  
characterized in that the weight ratio of primary  
emulsion to outer aqueous phase lies in the range 80/20  
to 40/60.

15       10/ An emulsion according to any one of claims 1 to 9,  
characterized in that the size of the primary emulsion  
droplets is less than or equal to 5 microns, and  
preferably less than or equal to 1 micron.

20       11/ An emulsion according to any one of claims,  
characterized in that the droplet size in the final  
emulsion lies in the range 1 micron to 200 microns.

25       12/ A dressing sauce or vinaigrette, in particular for  
salad, comprising an emulsion in accordance with any  
preceding claim.

30       13/ A dressing sauce or vinaigrette, in particular for  
salad, according to claim 12 and characterized in that it  
includes flavoring ingredients mixed in the emulsion.